



Microbial fuel cell: Critical factors regulating bio-catalyzed electrochemical process and recent advancements



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ARTICLE INFO

Article history:

Received 10 October 2013

Received in revised form

1 July 2014

Accepted 10 July 2014

Keywords:

Bioenergy

Bioelectricity

Bioelectrochemical System (BES)

Wastewater treatment

Electron transfer

ABSTRACT

Microbial fuel cells (MFC) are bio-catalyzed electrochemical hybrid systems which function by converting chemical energy to electrical energy through a cascade of redox reactions in the presence of biocatalyst. The research on MFC has been intensified in the last few years due to its inherent ability to produce sustainable energy from renewable organic waste. The current review depicts an overview on the fundamental operational mechanism of MFC encompassing electromotive force, electron delivery, electron transfer, losses encountered during operation, etc. The specific function of physical, biological and operational factors on the bioelectrogenic activity is elaborated. In addition, the strategies to regulate the process towards enhancing the performance of the system have been discussed. The potential applications of MFC for energy generation, waste remediation and value added product recovery have also been elaborated.

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1. Introduction

Microbial fuel cell (MFC) is a bio-catalyzed electrochemical system which can directly convert chemical energy from an organic substrate to electrical energy through a cascade of redox reactions [1–7]. The microbial metabolism is linked via electron donating and accepting conditions through the artificially introduced electrodes (anode and cathode), that induces the development of potential difference which acts as a net driving force for bioelectrogenic activity [8–11]. MFC can utilize a wide range of soluble or dissolved complex organic wastes/wastewater and renewable biomass as substrate that further offers the dual benefits of renewable energy generation in the form of bioelectricity with simultaneous waste/pollutant remediation, which makes the process eco-friendly [12–14]. MFC is gaining profound interest and importance in the present bioenergy research due to its innate potential and sustainable nature. Reports on MFCs were sparsely noticed from 1994, however considerable impact is being noticed since 2003 (Fig. 1) [15]. The citations also increased rapidly in the recent years indicating the

relevance and importance of the research on MFC. Studies on MFC using wastewater were more focused since 2004 and it was evident that wastewater is a potential substrate for bioelectricity generation. Research on MFC could pave a way in the field of renewable energy generation that could answer several complex environmental pollution problems and the energy crisis with a unified approach [5,6,10]. Apart from harnessing power, MFC has documented other applications viz., bioelectrochemical treatment system (waste remediation), bioelectrochemical system (bioelectrosynthesis of various value added products) and microbial electrolytic cell (H_2 production at lower applied potential) [6,7].

MFCs are featured simple, yet governed by various crucial parameters that regulate their performance. In an MFC, there exists a need to depend on several factors like physical, physico-chemical, chemical, biological, electrochemical, etc., which will essentially influence the rate of microbial electron transfer and power output [13]. Physical factors that significantly govern the MFC performance are of vital importance in MFC. The reactor configuration influences the biocatalyst activity and various

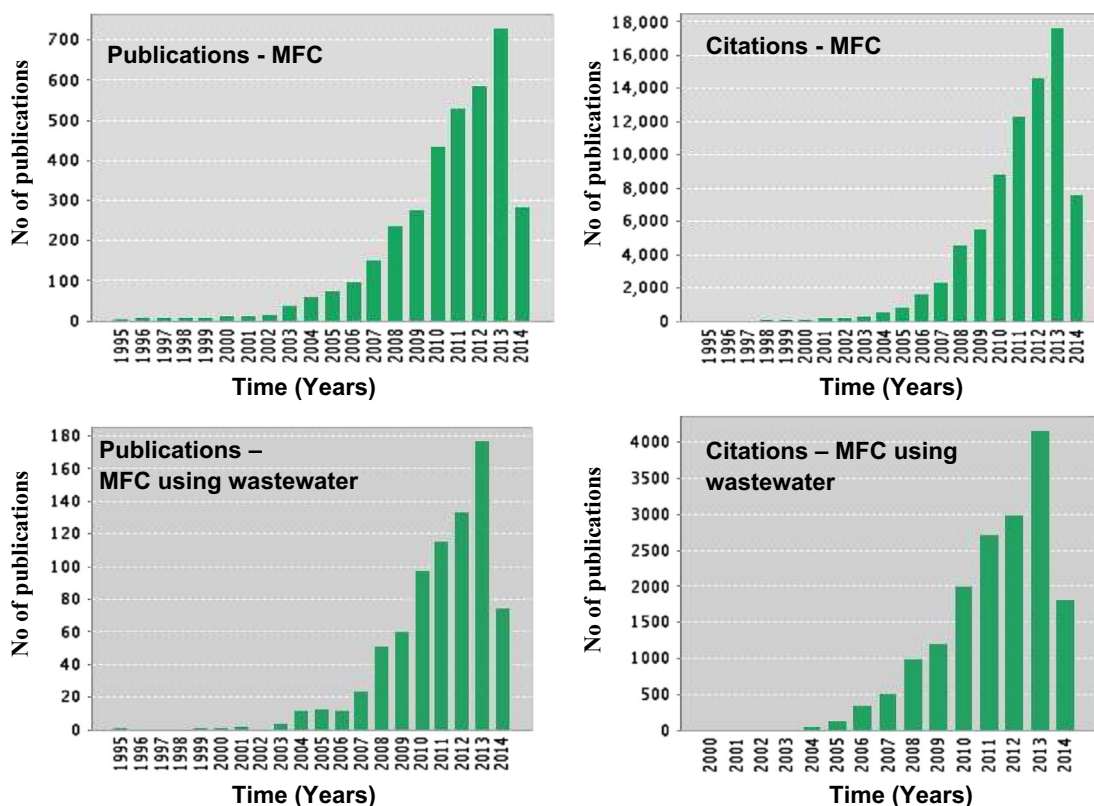


Fig. 1. Scientometric evaluation of the microbial fuel cell (MFC) research pertaining to the publications and citations (ISI Web of Knowledge; Keywords: microbial fuel cell and wastewater (as on 25th May 2013)).

Nomenclature

AHLs	acyl homoserine lactones	H ₂	hydrogen
ATP	adenosine-tri-phosphate	H ₂ S	hydrogen sulfide
Bchl	bacteriochlorophyll	HNQ	2-hydroxy-1,4-naphthoquinone
BES	bioelectrochemical systems	kH	kilo hertz
CAP	chloramphenicol	MB	methylene blue
CNT	carbon nanotube	MelB	Meldola's blue
CO	carbon monoxide	MET	mediated electron transfer
Co	cobalt	MFC	microbial fuel cell
CO ₂	carbon dioxide	mn (iv)	manganese IV
COD	chemical oxygen demand	mV	millivolt
Co-OMS-2	copper-octahedral molecular sieve	NAD ⁺	nicotinamide adenine dinucleotide
CoTMPP	cobalt(II) tetramethoxyphenylporphyrin	NR	neutral red
CP	carbon paper	O ₂	oxygen
DAO	direct anodic oxidation	OH [•]	hydrox radical
DET	direct electron transfer	OprF	porin
DSF	diffusible signal factors	ORR	oxygen reduction reaction
e [−]	electrons	PAH	poly aromatic hydrocarbons
<i>E. coli</i>	<i>Escherichia coli</i>	PAO1	<i>Pseudomonas aeruginosa</i>
EAB	electrochemically active	PbO ₂	lead oxide
EET	extracellular electron transfer	Pc	phthalocyanine
ETC	electron-transport chain	PDB	partially developed biofilm
FAD ⁺	flavin adenine dinucleotide	PHA	polyhydroxyalkanoates
FDB	fully developed biofilm (FDB)	PhFC	photosynthetic fuel cells
Fe (III)	iron III	PMF	proton motive force
Fe	iron	PQQ	pyrrolo quinoline quinone
FePc	iron phthalocyanine	QS	quorum sensing
FMN	flavin mononucleotide	RVC	reticulated vitreous carbon (RVC)
GF	graphite felt	SHE	standard hydrogen electrode
GNB	Gram negative bacteria	TEA	terminal electron acceptor
GPB	Gram positive bacteria	TiO ₂	titanium oxide
H ⁺	protons	TMPP	tetra methoxy phenyl porphyrin
		V	volt

studies pertaining to design and configuration of MFC were carried to elucidate its importance in power generation [12–14]. Operational factors such as nature and type of substrate, substrate load, pH, electrode materials, spacing between the electrodes and membrane materials etc., which influence the MFC performance were also studied [13,14]. The other major important factor is the biological factor, where the microbes act as biocatalyst to degrade the substrate that will also subsequently influence the electron transfer rate and bioelectricity generation. Biocatalyst, which is the core part of MFC will undergo diverse biochemical pathways and acquire various electron transfer mechanisms depending on the parameters designed for an MFC.

The current review made an attempt to provide an insight on critical factors that regulates the MFC performance based on the bio-electrogenic mechanism.

2. Mechanism of bioelectrogenesis

In 1910, Potter stated, 'the disintegration of organic compounds by microorganisms is accompanied by the liberation of electrical energy' [16]. Most probably this was the earliest observation of what we now know as exocellular electron transfer (EET), the process by which microorganisms transport electrons into and out of the cell from or towards an insoluble electron donor or acceptor. Microbes generally carry out their metabolic activities (anabolism and catabolism) either in the presence of O₂ (aerobic) or in the absence of O₂ (anaerobic) [6]. Irrespective of the nature of metabolism, microbes utilize the available substrate (fermentation) generating the reducing

equivalents [protons (H⁺) and electrons (e[−])] in the form of redox carriers viz., nicotinamide adenine dinucleotide (NAD⁺), flavin adenine dinucleotide (FAD⁺), flavin mononucleotide (FMN⁺), etc. These redox carriers help in generating energy [adenosine-tri-phosphate (ATP)] during respiration. During fermentation, reducing equivalents move through a cascade of redox components towards an available terminal electron acceptor (TEA). Thus a proton motive force (PMF) is generated which helps in the formation of energy rich phosphate bonds (ATP) useful for the cell growth and subsequent metabolism. The function of TEA is based on the thermodynamic hierarchy of the electron acceptors available in the system [6]. In the presence of O₂, which is having maximum reduction potential in the biological system and equally strong electro-negativity, the reducing equivalents pass through a redox cascade of respiratory/electron-transport chain (ETC) towards O₂ where ATP is generated through oxidative phosphorylation [6,17]. In the absence of O₂, other electron accepting molecules available in the system drive the electron flow through the redox cascade [6]. Thus, anaerobic metabolism provides possibility of harnessing the electrons available in the system into various forms of energy or valuable added products [12].

The prime function of MFC is based on harnessing the available electrons (e[−]) by artificially introducing electrodes as intermediary/terminal electron acceptors. Oxidation of substrate catalyzed by microorganisms takes place at the microbial (biotic) anode (Eq. (1)) which generates reducing equivalents, while reduction takes place at abiotic cathode (Eq. (2)). Protons cross the proton exchange membrane (PEM) and reach the cathode to generate a positive cathodic potential. The electrons remained at the anode generates negative anodic potential (Fig. 2). The overall reaction

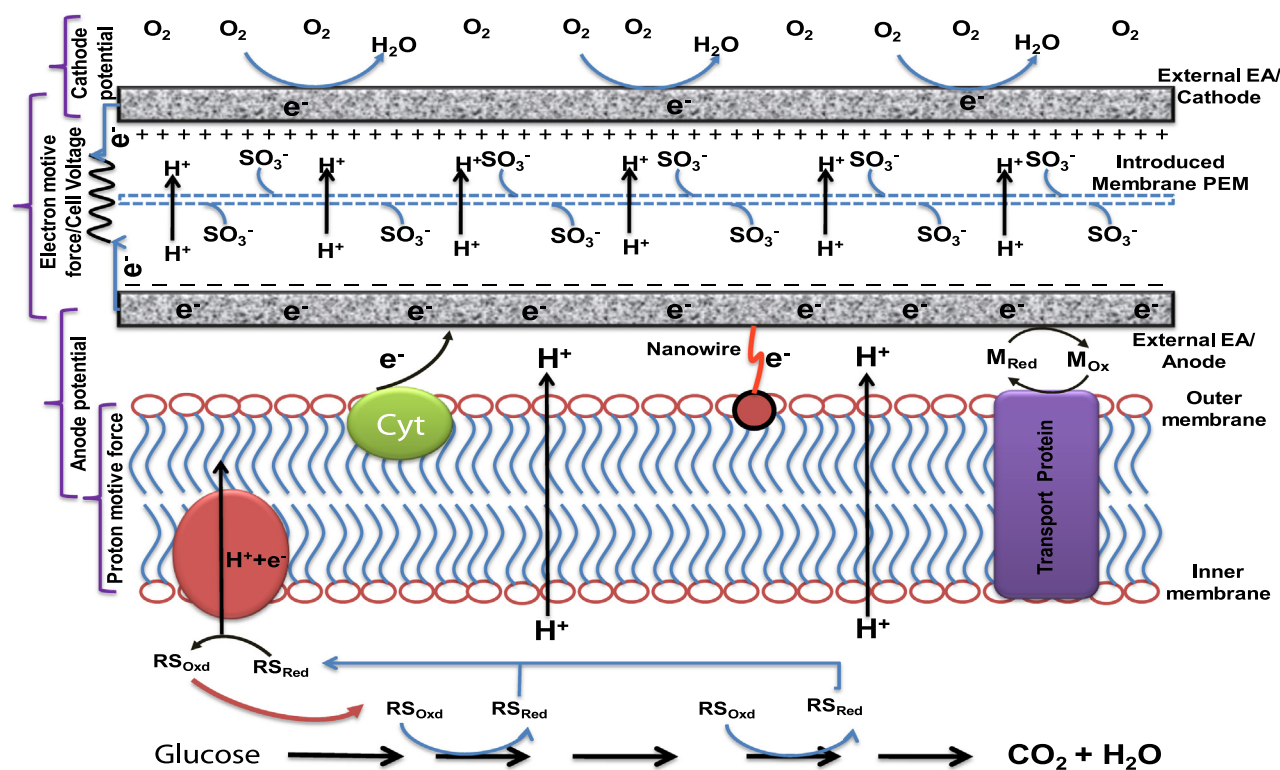
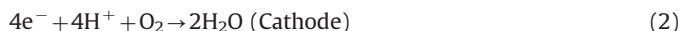
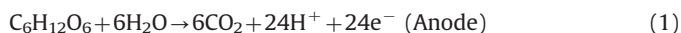


Fig. 2. Schematic representation of reducing equivalents generation and their transfer to TEA including the principle and mechanism of electron mobility from its source to sink.

involves the breakdown of substrate to carbon dioxide and water with a concomitant production of electricity as a by-product as represented by Eq. (3). The difference between positive cathodic and negative anodic potentials is considered as cell voltage/electron motive force, which drives the electrons from anode to cathode.



2.1. Electron motive force (emf)

Mitochondria are known to be the power house of a eukaryotic cell, in which major reactions take place with the energy generated through the membrane bound redox cascade of the mitochondrial electron transport chain (ETC). If the fuel cell is considered as eukaryotic cell with multiple functions, all the microbes present in it act as power houses of the cell performing diverse functions, viz., bioelectricity generation, waste remediation, value-added synthesis, etc. [5,7,18]. Each component of MFC has a specific and unique function. The electrodes act as redox towers of the cell assisting in the electron flow towards the TEA while the proton exchange membrane (PEM) introduced between anode and cathode mimics the function of an external membrane generating a potential gradient. Electron transfer from the source (metabolism) to sink (TEA) is driven by the potential difference between the redox active towers of MFC (Fig. 2).

The membrane potential across the cascade of membrane components is called proton motive force (PMF) due to which the reducing equivalents reach the inter-membrane space [6]. The

presence of anode connected to cathode via circuit has a deficit of electrons and needs to generate electrons continuously. This stress induces the development of potential difference between bacterial membrane and anode resulting in the generation of anode potential that helps in the electron delivery. This mechanism is termed as exocellular electron transfer where the electrons reach the anode to induce negative anodic potential, while the protons go to the cathode to induce a positive potential. Correspondingly, on the other side, a potential difference gets developed between cathode and TEA (cathode potential) [6,19]. The potential difference between negative anodic potential and positive cathodic potential is called electron motive force or cell voltage. This helps in the mobility of electrons from anode to cathode in the circuit across the load which can be harnessed as electricity [20].

The electron transfer from the source to a sink is mainly based on the differences in the redox potentials of the components of the fuel cell, irrespective of their nature (biological or chemical or physical) [6]. Lower anodic potential indicates the transfer of higher number of electrons into the exterior environment and less energy transfer towards microbial growth and cell maintenance. On the contrary, higher anode potential indicates higher energy transfer towards microbial growth which enhances the growth rate of bacteria. There is always a need for an optimal anode and cathode potentials which can suffice both cell growth and energy output [21,22]. However, for maximum electrical energy output, the anode potential should be as low as possible and the cathode potential as high as possible [23,24]. During the startup phase of MFC operation, the anode potential is generally high in favoring the bacterial growth rate which slowly decreases with time to increase the electron transfer against the cell emf/voltage. Anode potential can also be correlated to the metabolic pattern of the biocatalyst and energy dissipation [19,22].

2.2. Energy conservation and electron acceptor conditions

Energy generation in the form of adenosine-tri-phosphate (ATP) is manifested by proton motive force (PMF) induced by the mobility of reducing equivalents in the redox cascade towards the available terminal electron acceptor [6,25]. Respiration is an energy management process achieved through electron transport and simultaneous phosphorylation by means of external electron acceptors. The free energy available during redox reactions in respiration is conserved in the form of PMF, which is an interconvertible form of ATP. ATP synthesis in aerobic metabolism takes place via electron transport coupled with phosphorylation, while in anaerobic metabolism, ATP production is through substrate level phosphorylation, Na^+ -dependant decarboxylases, fumarate reduction and product/proton symport mechanism [25]. Oxygen is the readily available TEA in the aerobic respiration of prokaryotes, while anaerobic respiration can use diverse electron acceptors except O_2 .

The term anaerobic respiration is used to describe the energy conservation process using electron acceptors other than O_2 [6,25]. A diverse range of molecules can act as electron acceptors in anaerobic respiration, viz., oxidized sulfur and nitrogen compounds, metal ions, organic halogens, carbon dioxide, iodate, perchlorate, phosphate, etc. However, the selection of electron acceptor is naturally based on thermodynamic hierarchy, where the compound with higher redox potential is preferentially utilized over the compound with lower redox potential. For example, nitrates as electron acceptor (denitrification) will conserve more energy followed by sulfate/sulfur (sulfidogenesis) and carbon dioxide (methanogenesis). This results in inhibition of sulfidogenesis and methanogenesis in the presence of nitrate followed by the availability of sulfate/sulfur inhibiting methanogenesis. However, denitrifiers, metal reducers and sulfide reducers couple their respective reduction pathways with the generation of PMF having few exceptions that help in energy generation [24,25]. Physical separation of the anaerobic fermentation and respiration by introducing solid electron acceptors (electrodes) drives the electron flow out of the microbial cell that can be harnessed as bioelectricity in presence of different terminal electron acceptors. Compared to conventional biological treatment processes, treatment is more favored in MFC considering the pollutants present in wastewaters as electron donors.

2.3. Electron transfer mechanism

Microbes with high electron discharge capabilities are considered to be electrochemically active and are important for the MFC operation [2,6,26–28]. Electron transfer from the metabolic activities of the biocatalyst to the anode (intermediary electron

acceptor) is catalyzed by two major mechanisms, viz., direct electron transfer (DET) and mediated electron transfer (MET), based on the electron carrier involved (Fig. 3). The extra cellular electron transfer (EET) rate is influenced by the potential difference between the final electron carrier and the anode, regardless of the mechanism [24,29]. The cell compartmentalization and the highly complicated architecture of cell respiratory chains give an advantage to harness energy from biocatalysts [29]. Bacteria catalyze the decomposition of carbon sources by diverse anaerobic metabolic pathways in the anodic chamber of MFCs to generate intracellular electrons. These electrons are subsequently transferred to electrodes either via DET by redox c-type cytochromes present on the membrane / conductive pili or indirect electron transfer (MET) mediated by electron shuttles [30–34]. EET is a major limiting factor that governs the power output of MFC [34]. The electron shuttle-mediated EET is one of the most widespread electron transfer pathways in many microorganisms [31,33], such as *Shewanella oneidensis*, *Pseudomonas aeruginosa* [1,35], and *Escherichia coli* [36]. However, the bacterial outer membrane is often a low permeable barrier for the transport of electron shuttles across the cell membrane. This adversely limits the efficiency of EET and is responsible for the low power output of MFC [37].

One strategy to accelerate the electron shuttle mediated EET is to enhance the permeability of cell membrane [38]. This mechanism of enhancing membrane permeability is relatively easier in Gram negative bacteria (GNB) in comparison to Gram positive bacteria (GPB) due to inherent thin membrane structure [39]. *E. coli* when subjected to continuous stress for discharge of electrons form larger pores on its outer membrane and enhance the transport of endogenous electron shuttles across cell membrane to achieve more efficient EET [36]. Permeabilizers such as chitosan, ethylenediamine tetra acetic acid and polyethyleneimine were used to perforate on the bacterial outer membrane, which increases its permeability and the rate of secretion of electron shuttles, leading to an enhanced EET [37]. Membrane permeability is considered to be vital for an efficient EET [40]. A porin protein OprF from *P. aeruginosa* PAO1 was heterologously expressed into *E. coli*, which increased membrane permeability and delivered higher current output than its parental strain [38]. Mechanisms like quorum sensing (QS) and synergistic interactions among the bacteria also play a key role in aiding electron transfer for enhanced electrogenic activity. The signaling molecules such as acyl homoserine lactones (AHLs), peptides and diffusible signal factors (DSF) also enhance the extracellular electron transfer [39].

2.3.1. Direct electron transfer (DET)

Direct electron transfer (DET) takes place through membrane bound cell organelles or conductive nanowires with no diffusional redox species being involved in the electron transfer from the cell

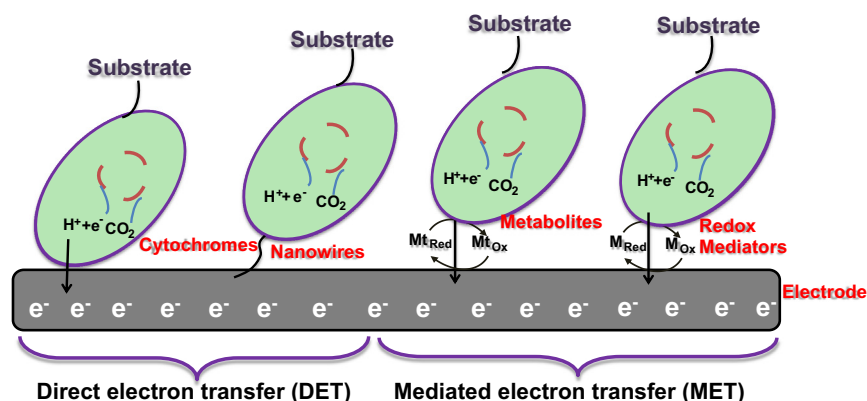


Fig. 3. Schematic representation of electron transfer mechanism from the microbial metabolism to the anode.

to the electrode [26,32,33,42]. DET is the physical contact of the bacterial cell with anode without involvement of any redox species or mediators. The bacteria should possess membrane bound electron transport proteins which could help in the electron transfer from the outer membrane of the bacterial cell to an external electron acceptor (anode). Studies have identified that a few electrochemically active bacteria have shown efficient DET mechanism, e.g., *Geobacter*, *Rhodospirillum rubrum* and *Shewanella* [2,33,43–46]. These microorganisms possess membrane bound electron transport proteins which transfer the electrons from bacterial cell to its outer membrane to an external solid electron acceptor/anode [33]. C-type cytochromes, multi-heme proteins are identified as one of the possible routes for DET. Major limitation of this process is that the bacteria should adhere to anode (in the form of biofilm) for the electron transfer. Many of the Gram positive bacteria are presumed to be involved in DET by biofilm formation through the teichoic acid which enables the adherence of bacteria on the electrode surface thereby providing a direct contact of membrane bound proteins with the electrode [39]. The only bacterial populations present in the first layer of biofilm are involved in the cytochrome mediated DET. Another form of DET is through the conductive pili (nanowire) formed on the bacterial cell surface connected to the cytochrome which can transfer/conduct electron flow from internal layers of biofilm to the anode [47–49]. Organisms like *Geobacter* and *Shewanella* are capable of producing electrically conductive nanowires [41,42]. This may allow the formation of thick electroactive biofilm leading to increased anode performances [50]. DET through bacterial nanowires is also possible through membrane bound cytochromes, due to the similarity in the redox potentials [49,51]. The function of proteins for DET irrespective of the bacteria was reported in literature [33,46,52–54]. Although a substantial amount of work has been done on DET mechanisms, a comprehensive evaluation on the proteins involved in the DET was not done yet.

2.3.2. Mediated electron transfer (MET)

Mediated electron transfer (MET) takes place through the redox shuttles that mediate the electron flow from the bacterial metabolism towards electrode. The outer layers of the majority of microbial species are composed of non-conductive lipid membrane, peptidoglycans and lipopolysaccharides that hinder the direct electron transfer to the anode. Electron mediators are found to accelerate the electron transfer [32]. Mediators in oxidized state can reduce in presence of electrons within the membrane. The mediators then move across the membrane and release the electrons to anode and become oxidized again in anolyte. This cyclic process accelerates the electron transfer rate and thus increases the power output. MET occurs either by the addition of artificial mediators or by secretion of soluble mediators such as primary and secondary metabolites from bacterial metabolism [33]. An ideal mediator should be able to cross the cell membrane easily, able to grab electrons from the electron carriers of the electron transport chains, should possess a high electrode reaction rate, good solubility in the anolyte, non-biodegradable, non-toxic to microbes and should be of low cost. A mediator with a higher electrode redox potential (E_0) would give a higher overall power than a mediator with the lowest redox potential [55].

Mechanism of MET represents an effective means to link bacterial metabolism to anode. However, the mechanism varies depending on the nature of the redox species. A wide range of substances belonging to the inorganic (potassium ferricyanide) or organic (benzoquinone) group facilitate electron transfer. Typical synthetic exogenous mediators include dyes and metallorganics such as neutral red (NR), methylene blue (MB), thionine, Meldola's blue (MeLB), 2-hydroxy-1,4-naphthoquinone (HNQ), and Fe(III)

EDTA [55,56]. The toxicity and instability of synthetic mediators limit their applications in MFCs. Anaerobic fermentation and respiration pathways facilitate the formation of primary and secondary metabolites that can serve as electron shuttles to the anode. Phenazines, phenoxazines, quinones etc., are the naturally secreted mediators [33,57]. Bacteria grown under soluble electron acceptor depleted conditions and at a distance from the solid electron acceptor (anode) tend to release low molecular weight electron shuttling compounds through secondary metabolic pathways, e.g., pyocyanin and ACNQ (2-amino-3-carboxy-1,4-naphthoquinone), etc. [58–60]. Pyocyanin released by *Paeruginosa* is well studied for its efficiency in electron transfer [61], which also supports electron transfer from other bacterial population to the anode [60]. Understanding the involvement of these metabolites is very interesting as their synthesis makes the process independent of soluble or solid electron acceptors. Usually, these shuttles are reversible in nature and therefore, they re-oxidize during electron discharge at the anode or at the soluble electron acceptor and are available for subsequent electron transfers [58]. However, the identification and evaluation of these extracellular electron shuttles is very difficult due to their low quantity. Pyocyanin and phenazine-1-carboxamide from *P. aeruginosa* [33,61] and quinone based redox shuttles from *S. oneidensis* [62] were reported. Moreover, the redox shuttles released by one organism can be utilized by other bacterial population. This synergetic interaction helps in the improvement of current generation in MFC operation as well as in the decrement of potential losses [1].

2.4. Electron losses

MFC operations undergo many electron losses during the transfer of electrons from the biocatalyst to anode that lower the conversion efficiency. The redox equivalents generated during substrate metabolism need to overcome many barriers prior to reaching anode and then cathode. During this process, there exist many possibilities of electron losses either due to neutralization or the acceptance by other electron acceptor, which is also termed as electron quenching [63,64]. Electron transfer from the biocatalyst to anode is influenced by internal resistances, otherwise known as potential losses and the electron transfer from the anode to cathode is regulated by external resistance. Especially at lower current densities, activation losses are considered to be crucial. Electron transfer is governed by many factors which include biocatalyst nature, fuel cell design, fuel cell components, operating conditions, anolyte nature, etc. The influence of external and internal resistances can be understood by polarization profiles (Fig. 4) and Tafel analysis which derives the active kinetic parameters that help to analyze and characterize fuel cell performance [64,65]. The electron discharge pattern of biocatalyst with respect to the external resistance as illustrated by polarization curve is plotted by considering the change in current density versus voltage and power density as a function of wide range of resistances. Decrease in resistance increases the electron flow through the circuit generating higher currents which lowers the potential difference between the anode and cathode. However, for achieving higher power outputs, both current and voltage should be high, as power is the product of both [6]. MFC attains a quick steady state at higher resistances, while it may take several minutes at lower resistances due to rapid drop in the cell voltage [63]. Increment in current and decrement in voltage continues as the external resistance decreases from infinite (where no electron flows through circuit) to zero (where maximum electron flows). For an ideal system, the power curve is parabolic in shape as the power output reaches a peak and drops down to a base. The resistance at which both the current and voltage are optimum resulting in highest power output is called the cell design point

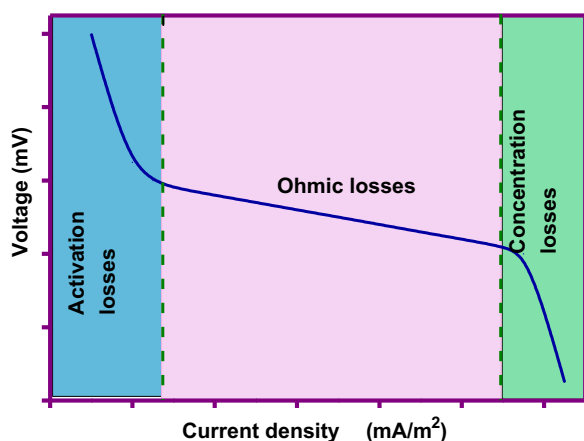


Fig. 4. Voltage curve of polarization against current density with the function of varying external resistance depicting the possible internal losses during the electron transfer from microbe to the anode.

(CDP) [64,65]. Operation of MFC below the CDP causes instability due to higher currents and lower voltages.

An S-shaped voltage curve is obtained for an ideal MFC where the early region depicts the activation over potentials, middle region depicts Ohmic losses and the terminal region depicts concentration losses [6] (Fig. 4). Voltage curve depicts internal losses of the system that hampers the electron flow from the biocatalyst to the anode. Oxidation at the anode or reduction at the bacterial surface or interior requires certain activation energy which incurs potential loss accounting for the activation over potential [66–69]. Energy required to carry out biological reaction is known as activation energy and all the reactants must cross an activation energy barrier to form products. Activation over potentials are important in lower current density zones (below 1 mA/cm²) [1] which can be minimized by increasing the operating temperature, anode surface area and concentration of redox shuttles [67]. Ohmic losses are caused by the electrical resistances of the electrodes, the solution-electrode interface and the electrolyte-membrane interface [65]. These losses occur where optimum voltage and current are generated. Controlling the Ohmic losses helps to harness higher power densities. Losses can be reduced by increasing the electrical conductivity of the electrolyte or by using highly conductive electrode materials. However, using noble metal electrodes such as platinum, titanium, etc. increases the economics. Wastewater as anolyte increases the conductivity of the electrolyte and can help in minimizing these losses. Concentration losses occur due to the large oxidative force of the anode, where the electron donor is oxidized at a faster rate releasing large concentration of reducing equivalents that cannot be carried to the anode and then to the cathode efficiently [67]. However, this is important at higher current densities where MFC becomes unstable. Thick and non conductive biofilm formed on the anode also hampers electron transfer to the anode surface. However, electrochemically active bacteria form a thin biofilm that aids in efficient electron transfer [68]. Apart from the aforementioned losses, electrons are also lost during competitive metabolic activities for the same precursor compound which is termed as electron quenching [67].

3. Factors influencing MFC performance

Electron discharge and power generation in MFC are mainly based on the potential difference between physical, chemical as well as biological components of the fuel cell. It is believed that

the bioelectrogenic activity of MFC is governed by several physical, biological and operational factors that essentially influence the power generation capabilities in MFC.

3.1. Physico-chemical factors

3.1.1. Electrode materials

Growth of electrochemically active bacteria depends on the active anode surface which accepts the electrons effectively from the substrate metabolism [6,46]. The synergistic association of the bio-anode with the biocatalyst plays a significant role in generating the electrons as well as their transfer towards the available TEA [69,70]. Therefore, it is imperative to select a suitable electrode material to minimize electron losses and increase the power generation efficiency of MFC. Electrode material used as bio-anode in the MFC should be electrically conductive, biocompatible, chemically stable in the anolyte, non-fouling in nature and should have high surface area with feasibility for large scale applications. It should also have efficient electron discharge properties, high porosity and sustainability over a period of time [6,45,69,71]. Platinum electrodes are highly electrochemically active and conductive in nature, but are too expensive. Stainless steel was also studied, especially in the sediment type fuel cells where good results were obtained in terms of power generation and remediation of organic contaminants [72]. Carbon based materials viz., graphite rod [73], graphite fiber brush [74], carbon cloth [75], carbon paper [76], carbon felt [52], reticulated vitreous carbon (RVC) [77] and carbon mesh [22] are most often used electrode materials due to their stability when microbial cultures (biocompatibility) are grown on them and also to ensure cheap process costs [69,72]. Pre-treatment of carbon based electrodes by ammonia [22,78] or chemical modification [79] or acid and heat treatment [71] was employed to improve the microbial composition in biofilm and to increase the electron transfer efficiency [80]. Plain and non-catalyzed graphite plates were used as anode/cathode [8,12,50] materials, keeping in view, the process economics.

Easy and effective colonization of the biocatalyst over the electrode surfaces and increased electron transfer are the two major criteria, irrespective of the material to be selected as anode [52,81–83]. Though graphite electrodes are cost-effective, the Ohmic resistance which is 1000 times higher than metals [83] and brittle nature, hinder its use for up scaling [69]. In some studies, the graphite electrodes were modified with surface coatings by electron mediators, active polymers, polyaniline and quinone groups to improve their performance [84,59]. Some researchers have utilized graphite or carbon in different forms such as granules, felt, foam, nanotubes and fibers in order to increase the anodic surface area [85–87]. Nano materials were also studied as anodic surface coatings due to their large surface areas, high mechanical strength, ductility and conductivity [88]. Carbon nanotubes in combination with a conductive polymer have been discovered as a potential combo material due to the diverse benefits it offer [36]. Graphite flakes were also used as supporting material for anodic electron transfer in the sediment type fuel cells which showed an increased adherence of bacteria and thus resulted in an increment in the volumetric power density [90]. Metal based materials like graphite, aluminum, brass, copper, nickel and stainless steel were also studied for their synergistic interaction with the biocatalyst in terms of electron discharge and microbial growth pattern. Copper, aluminum and brass were reported as non-suitable materials for anodes due to their solubility, easy oxidizing properties and its toxicity to the biocatalyst [69,89]. However, nickel and stainless steel can be used as a replacement of graphite, especially in large scale MFC operations [69]. Significant biofilm growth with enriched Gram positive

bacterial population was reported on the anode surface and long term sustainability of the electron discharge properties made these materials preferable [69].

Similar to the anode, cathode also has significant impact on power generation efficiency and it should also have high redox potential to confine the protons [91]. However, the electrode is an intermediate electron acceptor, delivering the electrons to the TEA yet, is also believed to constitute equal contribution towards power generation. Graphite [8–10,50,92,93], carbon cloth [94] and carbon paper [95,91], platinum [96], titanium [85], graphite foils [97], graphite plate [98], etc. were reported to be used as common cathode materials. Apart from these materials, graphite granules [99], granular activated carbon [100,101] and graphite flakes [90] were also studied in detail as the cathodic surface coatings. More recently, activated carbon obtained from various sources was also studied as cathodic surface coating [102]. A non-platinized, gas diffusion electrode with a stainless steel current collector, was also reported as air cathode [103,104].

3.1.2. Surface area of electrode and electrode spacing

The surface area of the anode also plays a critical role in MFC performance, as a larger surface area will provide more space for the microbial adhesion, resulting in increased electron transfer rates [88,90]. Similarly, placement of electrodes will also have a significant influence on proton–electron mobility associated with the power generation potential of MFC [65,73,85]. Considering the role of a charge carrier, a shorter diffusion length is believed to give fast electrochemical reaction due to the short diffusion time [65,73]. Maximum power output can be obtained by reducing the electrode spacing due to the consequence of reduced internal resistance [73,85]. The internal resistance in terms of Ohmic losses can also be reduced, by decreasing the electrode spacing [65,105,106]. However, placement of electrodes by smallest possible electrode spacing was reported to yield less power [85]. It is also believed that lower electrode spacing may allow the oxygen into the anodic chamber from the cathode which may increase the electron losses, but in the case of MECs, the electrode spacing can be too small since it involves in anaerobic reactions at the cathode [107]. Therefore, an optimum spacing is required between anode and cathode to enhance the electron acceptance from all over the reactor and to decrease the activation losses.

3.1.3. Nature of catholyte

Though the protons and electrons are generated and transferred to the cathode, their reduction rate in presence of an available TEA will determine the power generation efficiency of MFC. The electron acceptance capability varies among various available electron acceptors such as O_2 , Fe^{3+} , Mn^{2+} , etc. [8–11, 92,108]. Oxygen is the most readily available potential electron acceptor for biological systems, having a high reduction potential (+0.816 V) and is therefore most widely used. However, providing pure oxygen at cathode always is not feasible due to less economic viability. There are also studies using various electron acceptors such as Fe^{3+} , Mn^{2+} , etc. [8–11,92,108] but the necessity of a recurrent change in catholyte and the resulting toxic load on the the system microenvironment did not allow them to survive as potential catholytes. Ferricyanide showed higher reduction capabilities with respect to power generation and substrate removal efficiency in comparison to aerated catholytes [10]. This might be ascribed to the strong oxidizing nature of ferricyanide over oxygen/air along with the higher mass transfer rate and lower activation energy for cathodic reaction [71,109]. Other catholytes like CoTMPP [85], iron phthalocyanine (FePc) [110], manganese oxides [111], rutile (TiO_2) [112], lead oxide (PbO_2) [110], Co-OMS-2

[113], etc. were also studied. Compared to a single catalyst, a mixture of catalysts showed higher power generation.

Researchers also reported the use of bacterial metabolism as the TEA at cathode [65,69], especially the nitrifying and sulfate reducing bacteria [2,108,114]. In the recent studies, several organic and inorganic pollutants, viz., chloroorganics, azo dye, etc., were also studied as TEA at cathode [115]. Comparatively, aerated catholyte is easier in operation and the reduction reaction is self-sustaining with a limitation of requiring continuous energy input (for aeration) [116]. The evolution of single chambered open-air cathodes reduced the requirement of energy input to zero for the cathodic reduction reaction with their simple design. The efficiency of open-air cathodes is nearly 59% less than the ferricyanide catholyte [10] but the process is more eco-friendly in nature and can be easily adapted to the existing effluent treatment plants [12]. Moreover, the difference in substrate degradation is also not more than 8%, indicating trivial influence on anodic oxidation [10]. Using ferricyanide or other synthetic catholytes will significantly influence the reduction reactions that ultimately result in higher power generation. However, considering the economics and the toxic behavior of the synthetically supplemented catholytes, using open air cathodes in microbial catalyzed systems proved to be efficient in terms of economics (utilizes free atmospheric air) and to extend for large scale operations. Yet, the limitations in electron flow towards cathode due to the ineffective reduction reaction should be addressed to increase the columbic efficiencies of open-air cathodes.

3.2. Biological factors

3.2.1. Biocatalyst

Biocatalyst is the key component that governs the overall MFC performance through their substrate metabolism and exocellular electron transfer. Electron transfer may take place through membrane-bound proteins (direct electron transfer) or soluble shuttling compounds (mediated electron transfer) [117,118]. Different types of mono and mixed biocatalysts from various origins were studied for their efficiency of electron discharge in the microbial catalyzed electrochemical systems. It was reported that the enrichment of electrochemically active bacteria (EAB) on the anode surface will result in high power densities [19,22–23].

3.2.1.1. Monoculture. Several electrochemically active (EAB) and non-electrochemically active bacteria (non-EAB) were studied for their individual efficiency of electron discharge in MFC operation (Table 1). About all reported microorganisms viz., *Geobacter sulfurreducens* [119,120], *Rhodospirillum rubrum* [45], *Aeromonas hydrophila* [54], *P. aeruginosa*, *Pseudomonas otitidis* [59,61], *Geopsychronacter electrodiphilus* [121], *Desulfobulbus propionicus* [121], *E. coli* [59] *Rhodospseudomonas palustris* DX-1 [122], *S. oneidensis*, *Shewanella halotidis* [123,124] are electrochemically active, facultative and metal reducing bacteria. O_2 diffusion through the cathode into the anode chamber could affect the microbial activity, and therefore facultative microbes grow efficiently in anode chamber [125]. Metal reducing species have a special ability to act as self mediators, transferring electrons to the anode through physical contact. Outer membrane cytochrome oxidase type C protein allows the transfer of electrons from the inner bacterial cell membrane to the outer cell membrane. These microorganisms also generate nanowires to increase organism–anode physical contact thereby increasing the power density [47]. *Geobacter* and *Shewanella* strains are the most studied mono strains in this direction. However, the maintenance of the sterile, aseptic conditions and requirement of pure substrates for the strain growth and metabolic activities is one major stumbling

block for its application. MFCs operated with pure cultures are closed systems and mainly used for research purposes at laboratory-scale rather than used in industrial applications. Apart from bacteria, MFC was also operated using yeast (*Saccharomyces cerevisiae*) as the anodic biocatalyst [59].

3.2.1.2. Mixed culture. Mixed microbial communities obtained from different origins, viz., anaerobic and aerobic bioreactors, soil, etc., are studied as anodic biocatalyst. Contrary to the mono cultures, MFCs operated with mixed cultures are typically open systems and thus more closely related to the industrial applications and account for economic viability especially when wastewater is used as anolyte [132]. However, the microbial community of a mixed culture in MFC varies depending on the origin of inoculum, substrate availability, reactor design and other operating conditions [8,9,33,50,133]. The change of microbial community influences electron discharging efficiencies and thus MFC performance [100]. Mixed cultures showed higher power densities but low Columbic efficiencies due to the possible multiple metabolic reactions and increased mass transfer losses. Mechanisms like quorum sensing (QS) were reported to influence the bioelectrogenic activity when mixed consortia were used as biocatalyst [39,134]. Mixed culture proved to be a potent biocatalyst for electron delivery from the waste remediation.

3.2.2. Biofilm

The anode chamber/working compartment containing bacterial consortia of MFC supports the growth of biofilm-forming as well as planktonic microorganisms with both populations being capable of mediating exocellular electron transfer. A comprehensive review on electroactive biofilms by Borole and co-workers (2006) presented the significance of biofilm in the performance of bioelectrochemical system (BES). Most of the microorganisms in MFCs depend on exogenous [135–137] or endogenous [35] mediator molecules for electron transfer and a few can deliver the electrons directly onto the anode [41,50,52,86,69]. Planktonic microorganisms can perform the electron transfer only through mediators, whereas biofilm-forming microorganisms can transfer electrons directly from the cell envelope to the electrode or across the biofilm. DET has higher kinetics of electron transfer than MET [68]. Therefore, the ability of a microorganism to adhere to an electrode as biofilm plays a key role in increasing the power density of MFC.

The growth of microorganisms as planktonic cells or as biofilms is solely dependent on the metabolic status of the cell and

operating conditions. The activity and performance of biofilms formed on the electrodes is regulated by physical, chemical, biological and electrochemical parameters. Electroactive biofilms formed on the anode by electrochemically active microorganisms have many potential applications, including bioenergy generation and production of value-added chemicals [135]. Understanding the biofilm formation and its function in bio-catalyzed electrochemical cells helps to improve the bioelectrogenic activity. Direct conduction of electrons is possible through the biofilm matrix to the anode surface [72]. Various studies were carried out on the influence of biofilm formation on current generation [11,81] and the extent of biofilm formation on the anode surface also influenced the performance of MFC [11]. A biofilm configured MFC showed potential to selectively support the growth of electrogenic bacteria with robust characteristics, capable of generating higher power yields along with substrate degradation, especially operated with characteristically complex wastewaters as substrates. It is also proposed that during MFC operation, substrate is available at the outer layers of biofilm while the electrode is only available at the inner layer of the biofilm allowing the formation of thin and open biofilm that allows the migration of substrate without hampering the transfer of electrons to the electrode [22]. Though biofilm formation is critical in electron transfer and bioelectrogenic activity, thick deposition of biofilm hampers the electron flow. Hence, an optimum biofilm thickness will be suitable for achieving significant power outputs. Furthermore, the understandings on the electro active biofilms can be extended to other biofilm-mediated processes such as metal reduction, bioremediation, biosensors, biocorrosion and potentially the human-microbe interface.

3.3. Operating factors

3.3.1. pH

MFC performance in terms of power generation and substrate removal also varies with respect to pH conditions. Changes in external pH can bring alterations in several physiological parameters, including cytosolic pH (internal pH), ionic concentrations, membrane potential and proton shuttling [93,138–139]. pH plays a vital role in governing the metabolic pathway of the biocatalyst and operating the process at standard pH conditions could stimulate the microorganisms to achieve maximum system performance [36]. pH influences the efficiency of substrate metabolism, protein synthesis, synthesis of storage material and metabolic by-products [9,10,37–40]. In most of the cases, acidophilic operations showed higher performance compared to neutral and

Table 1
MFC operation with mono-culture as biocatalyst.

Name of the microorganism	Type of wastewater	MFC configuration	Reference
<i>Saccharomyces cerevisiae</i>	Glucose	Single chamber	[59]
<i>Shewanella putrefaciens</i>	Glucose	Single chamber	[90]
Co-culture of <i>Clostridium cellulolyticum</i> and <i>Geobacter sulfurreducens</i>	Cellulose	Dual chamber MFC Ferricyanide cathode	[126]
<i>Enterobacter cloacae</i>	Cellulose	U-tube MFC carbon fibers as cathode	[127]
<i>Rhodococcus</i> and <i>Paracoccus</i>	Glucose	Single chamber MFC air cathode	[128]
<i>S. oneidensis</i> MR-1	Lactate	Dual chamber MFC	[129]
<i>E. cloacae</i>	Malt extract, yeast extract and glucose	Dual chamber MFC	[130]
<i>G. sulfurreducens</i>	Sodium fumarate	Single chamber MFC air cathode	[72]
<i>Clostridium butyricum</i>	Starch	Dual chamber MFC	[131]
<i>Rhodospirillum rubrum</i>	Glucose	Single chamber MFC air cathode	[45]
<i>Shewanella putrefaciens</i>	Lactate	–	[52]
<i>Klebsiella pneumonia</i>	Starch and glucose	Dual chamber MFC	[132]
<i>Rhodospseudomonas palustris</i> DX-1	Simple to complex organic matter.	Dual chamber MFC	[122]
<i>Desulfuromonas acetoxidans</i>	Marine sediments	Single chamber	[80]
<i>Aeromonas hydrophila</i>	Wastewater with acetate	Single chamber	[54]
<i>Pseudomonas aeruginosa</i>	Glucose	Single chamber	[61]

basic operations due to the possible acidogenic pathways as well as higher proton gradient in the cell [138,139]. Moreover, extracellular electron transfers will be high under acidophilic pH due to the activity of intracellular electron carriers which will help in translocation of electrons from bacteria to outside of the cell. In the case of neutral and basic operations, reduction of H^+ during the substrate degradation is visualized leading to lower availability of H^+ and electrons [93]. The operation of MFC under neutral pH showed higher substrate removal [139] which is in correlation to the specific enrichment/growth of methanogens that relatively degrade substrate at a higher rate in comparison to other class of bacteria. Contrary to this, single chambered air cathode MFCs with mixed culture as biocatalysts showed higher current generation at an optimal pH of 8–10 [140]. The insitu buffers played a major role in system buffering capacity when pH was increased beyond 8 when pharmaceutical wastewater was used as a substrate [141,142]. Bacteria require a pH close to neutral for optimal growth but are strongly influenced by the cathodic reduction reaction [143]. Anodic pH might also be increased due to cathodic alkaline reaction, yet bacterial metabolism constantly produces weak acid compounds to maintain their intercellular pH [144]. Equimolar consumption of protons with electrons and O_2 in the cathodic reaction helps to regulate the anodic pH near neutral. The pH might increase if protons are not replenished through the membrane [143]. Biological as well as electrochemical reactions of the MFC play a crucial role in altering the pH of the electrolyte. Proton generation and consumption occur simultaneously, but biocatalysts endeavor to balance these changes in accordance with the initial pH [140]. Imbalances in system pH occur due to differences in anodic proton generating reaction and cathodic hydroxide-ion generating reactions which contributes to the potential losses, therefore leads to lower power output [145]. Addition of external buffers like carbon dioxide, carbonate and phosphate were also studied for highly balanced MFCs [145–148]. Torres et al. reported that current densities increased with an increase in buffer concentration as the active biofilms can be extended deeper than expected [89]. However, carbonate was proved to be more beneficial than phosphate in regulating pH since inorganic carbon is available in all natural waters and has higher diffusion coefficients in water that enable its transport through the biofilm [147].

3.3.2. Nature of anolyte and load

The type and nature of substrate significantly influences the system performance. Diverse kinds of substrates have been experimented and utilized as anolyte in MFC for power generation. Wastewaters such as chemical, distillery, pharmaceutical wastewater, dye, cellulosic, petroleum based, etc. have been tested [92,141,149,150]. Also, simple substrates such as glucose, acetate, domestic [151], dairy based [152], vegetable based [153], food based wastewater [154] etc. have been tested in MFC operation. One major limitation while choosing the substrate is its nature and biodegradability. Complex wastewaters contain a large quantity of organic carbon (chemical oxygen demand), which has high number C-ring structures that cannot be easily broken down into simple substrates. On the contrary, simple substrates readily get utilized by bacteria resulting in a large number of reducing equivalents aiding for enhanced system performance. Hence, choosing the substrate depending on the nature and type is critical for proper functioning of the system [8]. Other wastewaters generated from industrial or domestic activities function as good substrates due to the presence of large fractions of degradable organics. Residues like agricultural crops and their waste products, wood waste, food processing waste, aquatic plants, algae, and effluents produced in human habitats can all be used as fermentable substrates in MFC operation. In addition, substrate load has significant influence on the power densities and Coulombic efficiencies of MFC along with the natural influence on bacterial growth and biofilm morphology [103]. Higher organic load during initial phases of operation leads to higher substrate removal but results in lower Coulombic efficiencies. This can be attributed to the utilization of organic matter for other microbial processes towards growth and physiological balances generating other by products through integrated approach [148,155].

3.3.3. Configuration

MFC systems are fundamentally designed with dual and single chamber configurations (Fig. 5). Dual chambered or H-type MFC is a conventional MFC for research in the initial stages [8]. A typical two compartment MFC has an anodic chamber and a cathodic chamber separated by a proton exchange membrane (PEM), or sometimes a salt bridge, to allow protons to move across to the cathode while blocking the diffusion of oxygen into the anode.

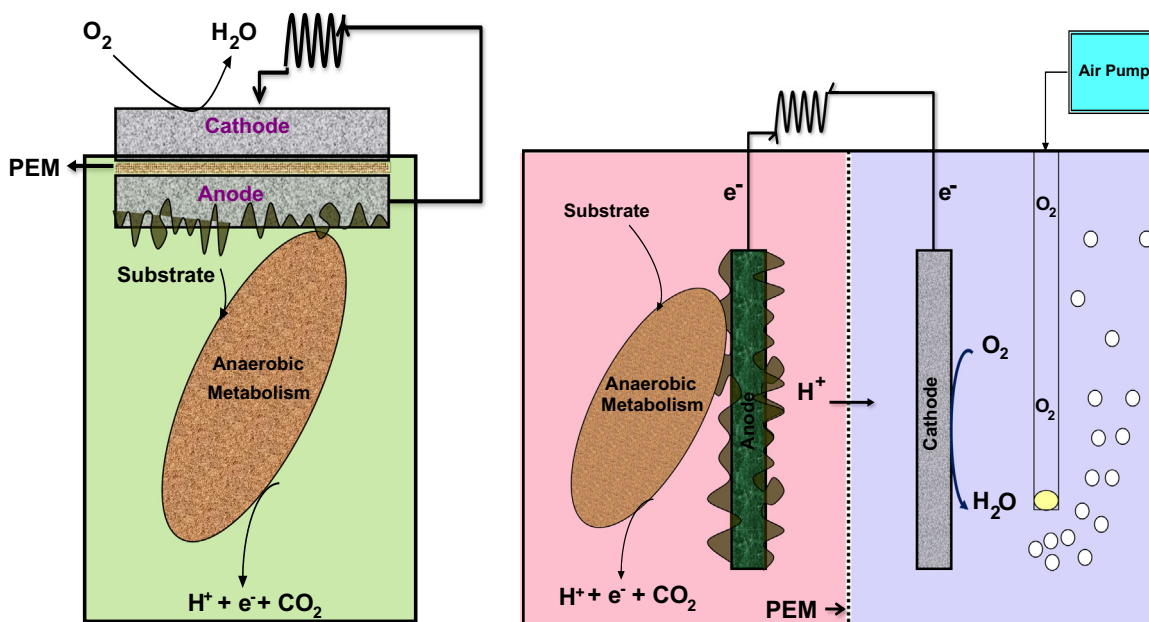


Fig. 5. Schematic representation of single and dual chambered MFC.

The compartments can be designed in various shapes [50]. The substrate gets oxidized in the anode chamber and the reduction reaction occurs in the cathode chamber. Catholytes such as potassium ferricyanide, potassium permanganate, aerated catholytes, etc. can be used with variable degrees of efficiency [138]. Whereas, the single chamber MFC configuration consists only the anode chamber, while cathode is placed in such a way that it is exposed to air (open air cathode). Double chamber MFC performance proved to be better in terms of current generation and treatment efficiency in comparison to single chamber MFC. The observed lower current generation, might be due to its own limitations during the cathodic reaction. Apart from these basic configurations, multi electrode assemblies in a single chamber or stacking two/three MFCs, U-tube MFC, serpentine MFC, etc. have also been studied (Table 2). In spite of the above work, researchers worked extensively on single chamber MFC, as the configuration is appropriate and viable for full scale operation and also for easy up-gradation in the existing conventional wastewater treatment units [71]. MFC performance is independent of the volume of anolyte because the possible theoretical potential is around 1.2 V [NAD^+ (-0.32 V) and O_2 ($+0.816$ V) as electron donor and acceptor in the biological system]. Therefore, to enhance the power output, stacking of fuel cells could be a good option.

4. Regulating bioelectrogenic activity towards improved process efficiency

Electrochemically active consortia will have higher membrane potential, which helps in delivering electrons against the anode potential. Therefore, selectively enriching the electrochemically active consortia on the surface of the anode helps to increase the electron delivery from the biocatalyst to the anode resulting in a higher power output [19,23]. Various methodologies were reported for the enrichment of electrochemically active consortia viz., application of poised potential, growth in mediator deprived conditions, bioaugmentation, etc.

4.1. Microbial anode

Anodic microenvironment influences the nature of biocatalyst metabolic activity and thereby affects the resulting redox reactions [8,141,161–163]. This also has a distinct influence on the nature and composition of the microorganisms enriched. The type of fermentation and respiration forms a major basis for the differences in metabolism under varying microenvironments. In general, the substrate is being oxidized at the anode (fermentation) generating the reducing equivalents which are further reduced at the cathode during respiration. Most of the MFC operations are based on the anaerobic metabolism at anode generating the reducing equivalents which get reduced at cathode by oxygen as terminal electron acceptors. However, there are few studies reported based on the aerobic and anoxic microenvironments in which, anodic oxidation is coupled to the cathodic reduction [8,86]. Anoxic microenvironments also had the capability to generate power under defined conditions. The usage of wastewater as substrate, where high substrate removal is accompanied with power generation [8].

4.1.1. Poised potential

Electrode potential is considered to be an essential factor regulating the process efficiency of a biocatalyzed systems or MFCs. The selection of anode potential is one of the key factors controlling synergistic interaction between the anode and the biocatalyst which influences the electron liberating capacity of the biocatalyst [23,33,69]. Anode potential determines the theoretical

Table 2

Bio-electrogenic activity with the function of fuel cell configuration.

Configuration	Power Output	Reference
Double chamber	66.21 mW/m ²	[50]
Double chamber	170 mW/m ²	[140]
Double chamber	1.3 mA/cm ²	[130]
Single chamber	305 mW/m ²	[73]
Single chamber	211 mA/m ²	[8]
Single chamber	150 mW/m ²	[151]
Single chamber	269 mW/m ²	[156]
Single Chamber	182.85 mA/m ²	[9]
Single chamber	354 mW/m ²	[156]
Benthic MFC	35.08 mW/m ²	[93]
Single chamber	107.89 mW/m ²	[154]
Single chamber	401 mW/m ²	[157]
Flat plate MFC	72 mW/m ²	[151]
Flat plate plant MFC	5.8 W/m ³	[158]
Stacked MFC	258 W/m ³	[100]
Basic stack MFC	1184 mW/m ²	[159]
Miniature floating macrophyte based ecosystem (FME)	224 mA/m ²	[160]

energy gain for the biocatalyst from a thermodynamic point of view, neglecting the metabolic pathway it undertakes [23,33]. Generally, the microorganisms at the anode adapt their electron transferring system to a level just below the anode potential [23]. Hence, it is believed that regulating the anode potential satisfies both microbial growth and electrical output during MFC operation [22,23]. Studies reported that, poisoning external potential to the anode or to the whole cell will help to enrich electrochemically active bacteria that significantly influence the fuel cell performance [19,23,33,60]. Optimized anode potential also helps in the early startup of electron discharge from the biocatalyst resulting in a larger current output [19,69]. Adhesion of the biocatalyst to the anode depends on the surface charge and other properties of the anode and biocatalyst [79]. Positive and negative anode potentials play a significant role in the electron liberation and enhanced performance. Application of a positive potential to the anode will increase the surface positive charge of the electrode enabling the adhesion of negatively charged bacteria [69]. Moreover, the positive anode potential can increase the energy yield per equivalent substrate oxidation. Poisoning optimum potential enables the rapid biofilm formation which leads to higher bioelectrogenic activity. Negative potential also aids in the synthesis of value added products or chemicals in the cathode chamber by the application of suitable potential that meets the effective reduction reactions in bioelectrochemical systems [14]. Applying negative anodic potential was also reported in few studies that showed the enrichment of *Geobacter sp.* on the anode surface with increased electrical output [161].

Understanding the growth, kinetics, and interactions of anodophilic microorganisms are the fundamental aspects of the bioanode [105]. At an anode potential of 0.04 V (vs. SHE), thin heterogeneous organisms form biofilm on the electrode [163]. Applying a positive anode potential reflects the growth of pure electro active organism (0.095–0.595 V (vs. SHE)) [124]. Optimizing the potential in anode is a feasible approach for efficient recalcitrant compound removal and higher power generation [164]. The application of a positive potential to the whole cell during the startup phase aided to enhance the performance in terms of power output and wastewater treatment [19,60]. On the contrary, some studies have stated that poised potential may have less effect on the enrichment of biocatalysts on the anode, but the electron discharge efficiencies were found to vary with the poised potential [22,23]. Some studies reported negligible influence of poised anode potential on the performance of the MFC [22,23] while the others reported its positive influence and even identified

new redox couples. The application of the poised potential influences the microbial dynamics and therefore can increase the electrogenic activity of the MFC by the selective enrichment of the electrochemically active bacteria [60,161]. Prolonged application of poised potential depicted a clear variation in the distribution and composition of the microbial community in which *Proteobacteria* were found dominant, followed by *Firmicutes* [60]. The presence of dominant bacteria known as recalcitrant organic degraders and/or exoelectrogens/electrotrophs includes *Desulfovibrio carbinophilus* and *Dechlorospirillum* sp. on the bio anodes showed enhanced performance at 200 mV [164]. MFC systems linked in series by dynamically applying potential in the kHz frequency range were also studied [165].

4.2. Cathode performance

The terminal reduction reaction which occurs at the surface of cathode is considered to be critical during microbial catalyzed fuel cell operation, as the fate of reaction depends ultimately on the cathode. The reduction reaction at the cathode also plays a significant role in the power generation efficiency of MFC. The material used for the cathode contributes about 47–75% of the capital cost of MFC and hence it is a pre-requisite to choose economically viable materials [102,143]. Research on cathodes gained considerable interest [64,65,102]. The electron losses occur at the cathode surface as well as in the anode compartment due to over potentials. Although small current might flow through the electrode surface, these losses also need to be considered due to the efficiency of MFC at limited ranges [64,65]. The electron acceptor (oxygen) concentration at cathode gets increased, the electron transfer efficiency also increases [66]. However, the use of pure oxygen is not feasible for the practical design. Apart from oxygen, potassium permanganate and ferricyanide were also used in the aerated catholyte to increase the reduction reaction [9–11,90]. However this requires recurrent change of catholytes and also adds toxic load on the environment. To limit the large cathode over potentials, various catalysts such as cobalt and iron tetra methoxy phenyl porphyrin (TMPP) or phthalocyanine (Pc) [94], and activated carbon were studied at cathode [166,167,97,102]. Similarly, noble metal electrodes such as platinum were also studied as cathode material which helped to decrease the activation over potential [102] but, this is an expensive material and has a limited resource. Moreover, the protons and electrons accepted from anode helps in higher substrate oxidation and remediation of pollutants in the cathodic chamber. In the absence of oxygen, nitrate, sulfate, fumarate and carbon dioxide act as terminal electron acceptors during anaerobic biocathode operation.

4.2.1. Biocathode

Conventionally MFCs were operated with biotic anodes and abiotic cathodes. Abiotic cathodes have disadvantages such as requirement of a chemical catalyst with noble or non-noble catalysts for oxygen reduction to meet the operational sustainability [164]. These problems could be overcome by using bio-cathodes, which use microorganisms as biocatalysts to assist cathodic reaction enhancing economic viability and environmental sustainability [164,168]. The mechanism of a biocathode is similar to bio-anode, but the components operate at different redox potentials and the biocatalyzed cathodic reactions are not necessarily energy conserving for the microorganism. The fate of any electro-chemical reaction focused towards bioelectrogenesis/remediation/synthesis of value added products depends on the cathodic reaction mechanisms as the final TEA appears at cathode.

Microorganisms can also be used as catalysts at cathode for the effective reduction process. Microbial growth is inevitable in the

cathodic compartment because it is not feasible to operate it as a sterile unit [169]. The biocatalysts retrieve electrons directly from the cathode [170] which are then transferred to a final electron acceptor such as oxygen, nitrogen, sulfur, etc. [171]. Literature reports are available on the increased performance of MFC with a cathode inoculated with microorganisms [170,171]. These studies suggest that oxygen reduction on the cathode was directly catalyzed by the biofilm formation. A few other researchers used specific metal reducing bacterium (Mn^{+2} , Fe^{+2} , etc.) in the cathode chamber which reported higher performance than abiotic cathodes [172]. Biocathodes have a potential advantage of reduction of pollutants such as nitrates or sulfates or chlororganics in the cathode compartment which could make this application more feasible when operated with wastewater [170,172]. The microbial reduction of metals such as Fe (III) and Mn (IV) and the treatment of nitrogen and sulfur rich wastewaters where they act as terminal electron acceptors in the cathode is an added advantage. Oxygen is the potential terminal electron acceptor during MFC operation and in the aerobic biocathodes oxygen acts as the oxidant assisting the microorganisms in the oxidation of metal compounds. Aerobic, anaerobic and microaerophilic (anoxic) microenvironments were studied in the MFC [20,64,65]. Higher power generation was observed with the aerobic biocathode operation. The significance of cathodic reduction on recovery of specific compound/chemicals has been studied using either mono/mixed culture [64].

Biocatalysts used in the biocathode produces useful products and remediates toxic pollutants [172]. Another solution is to use biofilms on cathodes for catalysis. In addition to aerobic biofilms that catalyze oxygen reduction, anaerobic biofilms can also be used to reduce a non-oxygen oxidant such as sulfate and nitrate that function as terminal electron acceptors in the absence of oxygen [141]. The advantage of using an anaerobic biocathode instead of an aerobic biocathode is the elimination of oxygen diffusion into the anode and preventing the loss of electrons in neutralizing the water molecules [173]. Feasibility of microalgal biocathode based MFC operation was reported taking into account the synergistic association between bacterial fermentation at anode and the oxygenic photosynthesis of microalgae at cathode which facilitated good power output as well as treatment efficiency [174]. Oxygenic photosynthesis by microalgae at cathode helped to maintain higher DO and thus eliminates the need of energy intensive aeration. Characteristics and configuration of biocathode materials are the major factors influencing performance of MFC [167]. Studies reported that compared to carbon paper (CP) and stainless steel mesh (SSM), graphite felt (GF) exhibited effective electrochemical performance viz., power generation, polarization, oxygen reduction reaction (ORR), higher catalytic activity and higher Coulombic efficiency [175]. Biocathode performance in dual chambered operation especially at higher resistance documented high power generation [176]. Although biocathodes are attractive because they allow the use of inexpensive non-catalytic electrode materials and they can also treat a second wastewater stream, the voltage output of an MFC with a biocathode can be much lower than that using an oxygen or air cathode. The oxygen reduction potential is far more positive than the reduction potentials of sulfate, nitrate, etc.

However, there are still several constraints which should prevail prior to making the biocatalyst favorable for future MFC applications. Research on biocathodes is presently in its infant stage. Major constraints that should be addressed are cathodic activation over potentials [171], dynamics of environmental factors [169], accumulation of metabolites and ions crossed over through the membrane and competition between metabolites generated in the cathode and anode as electron donors [169]. Biocathodes also provide a chance for the removal of certain pollutants as well as synthesis of value-added products.

Polyhydroxyalkanoates synthesis was demonstrated in a micro-aerophilic cathodic microenvironment [65]. Microbial metabolism at the biocathode addresses some of the major limitations faced by the scientific community such as long term operation, stable power production for longer periods, additional treatment, synthesis of value added products, etc., to make this technology as future fuel source.

4.3. Mediators

Mediated electron transfer (MET) is also one of the significant factors that influence MFC performance. Though DET is considered to be more efficient electron delivering system due to the lower mass transfer losses, it alone cannot deliver high magnitudes of power generation [2,33,8]. An electron carrier transfer electrons through a series of redox reactions. Mediators are the endogenous electron shuttles that carry the electrons towards the electrode. Mediators can be either secreted by the organism itself or can be added externally into a fuel cell which enhances the system performance by aiding the electron transfer effectively. The function of mediators is specifically important for the electrochemically inactive microbes, due to their inefficiency in electron delivery to the anode. This is due to electrically non-conductive cell walls and the obstruction from the peptide chain adjoining the active redox center of proteins [1,33]. Such inefficiency is circumvented in MFCs by either adding artificial electron mediators or by selecting either specific bacterial strains that can produce electron mediators or bacterial strains that have a strong ability to transfer electrons directly [1,33]. The first instance of introducing an electromotive redox active species (mediator) was found in 1930 by Cohen through the addition of potassium ferricyanide and benzoquinone to facilitate the electron transfer from biocatalyst to the immersed electrodes [177]. However, the approach was then studied in detail after 1980 by several other research groups across the globe [33]. A large number of artificial compounds were investigated for their suitability and behavior as MFC mediators but a majority of those compounds were based on phenazines, phenothiazines, phenoxazines and quinones [12,33,178]. The major disadvantage of using artificial redox mediators is the necessity of a regular addition of the compound, which is technologically not feasible and environmentally toxic [33]. Some bacteria, like *P. aeruginosa* and *Desulfovibrio vulgaris* produce their own mediators such as pyocyanin, pyoverdine, etc., [1,12,52]. Physical entrapment of the metal ions on the electrode was also studied as an option for the effective electron recovery, where 1000-fold increment in electrode efficiency was reported using an anodic electrode plate containing a fixed Mn^{4+} mediator [179]. Mediators may be exogenous or endogenous in function but they should possess some specific characteristics such as reversible nature, readily reduced, not metabolized by the biocatalyst, stable and soluble in reaction media over long periods of time, etc., to function as an effective electron shuttle.

4.4. Bioaugmentation

The power generation capacity of MFC depends on the catabolic activity of the anodic biocatalyst and its electron transfer efficacy to the anode. However, the transfer of electrons between the biocatalyst and the solid surface of the microbial anode (electrode) is low because of slow kinetics which subsequently results in low power yield [180]. The use of mixed consortia as biocatalyst and wastewater as feedstock with MFC makes the whole process economically viable. However, mixed culture contains a significant number of diverse bacterial species which may be EAB or non-EAB that lower the rate of electron transfer kinetics. Multiple metabolic interactions resulting between the mixed

consortia at times capture the electrons released by one species towards the growth of another species or neutralization that results in lowered performance. Various strategies have been proposed in literature to enhance the electron transfer rate between the biocatalyst and the electrode as discussed previously in this review. Bioaugmentation application was well known in waste bioremediation treatment and fermentation processes [47,181]. Bioaugmentation of EAB to the native consortia is one such strategy used to increase electron transfer efficiencies through the syntrophic association of augmented strains and the native culture [62]. Bioaugmentation of the electrochemically active *S. haloties* strain to the native anodic consortia was studied in MFC to evaluate the relative electron transfer efficiencies of *S. haloties* in comparison with native culture and bioaugmented microflora [62]. The rationale behind bioaugmentation application to MFC is to catabolically augment relevant organism having specialized characteristics that can improve the electron transfer efficiencies and thus the power output [62]. Robust strains containing desired characteristics that can persist for longer time in a habitat would have greater opportunities to transfer the desired characteristics to other microorganisms [47,181]. *Shewanella sp.* are exoelectrogens capable of producing electron shuttles, which permit them to reduce insoluble metal oxides despite lacking direct cell–electron acceptor contact [67]. Application of bioaugmentation resulted in two-fold higher power output compared to the native culture along with apparent increment in the energy levels to ten-fold [62]. The oxidative capability of the native culture and the augmented culture remained more or less same, but the electrogenic activity increased showing the decrement in the mass transfer losses between the biocatalyst and the electrode. The augmented culture showed higher performance than the pure culture supporting the syntrophic association of *S. haloties* with anodic mixed culture. Bioaugmentation studies were carried out to evaluate comparative performance of EAB (*P. aeruginosa*), non-EAB (*E. coli*) and native mixed consortia [134]. Long-term persistence of the augmented strain in the targeted system will have greater opportunities to transfer the desired characteristics to native microorganisms.

5. Multi-facet applications of MFC

Bio-catalyzed electrochemical mechanism occurring in MFC during operation provides inherent advantage to use it for diverse applications in the arena of energy conservation and value-added product synthesis [7]. The reducing equivalents (e^- and H^+) generated as a result of substrate metabolism will be utilized towards harnessing of power in MFC, waste remediation in bioelectrochemical treatment system (BET), bioelectrosynthesis of various value added products in bioelectrochemical system (BES) and H_2 production in microbial electrolytic cell (MEC) at lower poised potential (Fig. 6). MFC has gained considerable interest due to its ability to harness power from a wide range of substrates (anolyte fuel) which includes biomass, real-field wastewater, synthetic medium, etc. as electron donor [1,3,9,10,50,182]. The application of MFC in integrated strategies towards sustainable bioenergy generation is current interest [155]. MFCs are specifically designed to harness bioelectricity from benthic aquatic eco-system using natural habitats wherein, ecological water bodies are considered as a potential source to convert the organic rich sediment to energy [183,184]. Plant based MFCs helps in the utilization of solar radiation to generate bioelectricity by integrating the rhizodeposits of living plant with electrodes [183]. The scope of MFC application has been extended to the development of photo-electrocatalytic fuel cell using photosynthetic organisms as anodic biocatalyst to assess their potential to harness power [185].

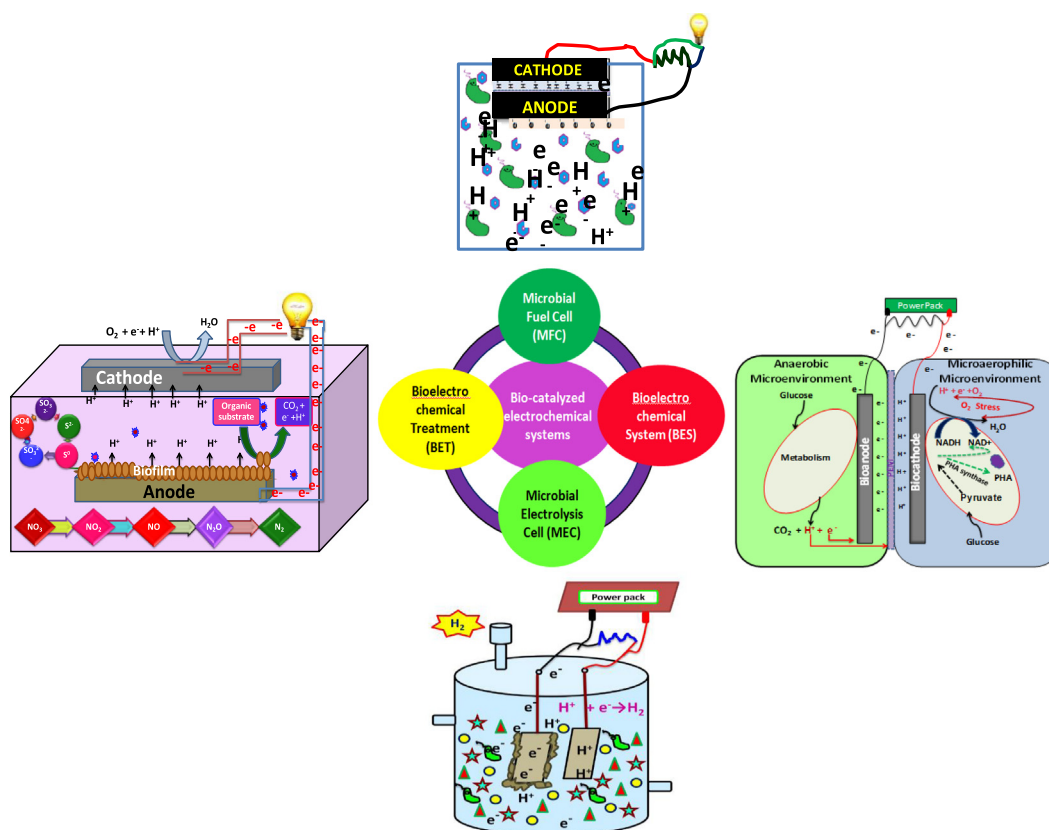


Fig. 6. Multi-facet applications of Bio-catalyzed electrochemical systems.

Apart from harnessing power, MFC when operated with waste as anolyte illustrated significant treatment efficiency and hence are termed as bioelectrochemical treatment (BET) system when the focus is towards remediation of waste [6,155,161,186–189]. BET can utilize soluble or dissolved complex organic wastes/wastewater as substrate and can degrade the pollutants that serve as electron acceptors during the operation and also help to lower the operational cost of effluent treatment plant. Bioelectrogenic activity occurring during BET operation facilitates the possibility of integrating diverse processes viz., biological, physical and chemical in anodic chamber which trigger multiple reactions [152,155]. In addition to organic substrate reduction, BET is effective enough for the reduction of pollutants such as nitrates, sulfates, phosphates, total dissolved solids (TDS), metals, perchlorate, estrogens, poly aromatic hydrocarbons (PAH) etc., can act as electron acceptors during the electron transfer [88,113,141,187,190–192]. In thermodynamic hierarchy, nitrates are considered as best electron acceptors followed by O_2 responsible for denitrification through dissimilative process where they act as electron acceptor [12,71,109,110]. Few microbes and archaea utilize sulfate as electron donor for the reduction of sulfur containing toxic pollutants to the elemental sulfur form which acts as a mediator further in the electron transfer process [34,86]. Toxic halogens and other hydrocarbons can also act as electron acceptors for anaerobic respiration that are recalcitrant to aerobic remediation. Colored compounds present in wastewaters such as distillery and pharmaceutical wastewater were also reported to act as redox mediators during the electron transfer [10,11,71,94,101].

In microbial electrolysis cell (MEC), the external potential applied in the system facilitates e^- and H^+ to cross the endothermic barrier to form H_2 gas. H_2 yields are comparatively higher than

the dark fermentation process and water electrolysis in MEC system [79,193]. Usage of membrane in MEC system prevents the reaction between H_2 and O_2 thereby increase the process efficiency by enhanced recovery of H_2 . Carbon nano tube (CNT)-based cathode materials and solar-powered MEC with a Platinum (Pt) catalyst were reported for the production of hydrogen gas [194]. Bioelectro synthesis (BES) is the processes in which electrically driven reduction of carbon dioxide occurs to synthesize chemical compounds viz., acetate, ethanol, hydrogen peroxide, butanol, etc. in an electrochemical cell [195]. The redox potential of the system determines the product formation based upon the electron acceptor conditions [195]. Value added product recovery at the cathode can be harnessed at lower redox potential sufficed by the *in situ* potential in the system *in situ* potential. Some reactions require additional redox potential at cathode which cannot be accomplished by the *in situ* potentials and hence an external potential is applied to meet the energy necessary to cross the energy barrier for product formation. Ethanol can be formed at cathode at a redox potential of -0.28 V by considering acetate as electron acceptor. Lab-scale studies have demonstrated the use of acetogens that have the ability to convert various syngas components (CO , CO_2 , and H_2) to multi-carbon compounds, such as acetate, butyrate, butanol, lactate, and ethanol, in which ethanol is often produced as a minor end product [14]. Microaerophilic biocathode in BES showed enhanced electrogenesis and simultaneous polyhydroxyalkanoates (PHA) production [196,197]. BES application can be extended to biosensors where the biocatalyst in the anode chamber acts as a biological detector to evaluate the performance of the system [198,199]. Micro-MFC for high-throughput screening and sensitivity analyses of biological and electrochemical performance parameters were also developed [200].

6. Challenges and future outlook

Microbial fuel cell (MFC) is being considered as a renewable energy generating system to combat the existing energy demand and pollution problems in an integrated and sustainable approach. Despite the major advances made, MFC still face considerable challenges apart from low power output which needs considerable attention. A comprehensive understanding of various critical factors that can regulate MFC performance has been depicted in the present review. Factors that are critical to fuel cell operation need to be exploited to accomplish effectual system performance. Electron losses that are encountered during fuel cell operation are a major hindrance that limit the power output. Hence, thorough understanding of the biocatalyst capabilities, electron transfer mechanisms and kinetics is obligatory.

6.1. Biocatalyst

Performance of the microbial catalyzed electrochemical systems is dependent on the efficiency of the electron transfer machinery. Power output (in MFC), quantity of pollutant treated (in BET) and nature of value added product synthesized (in BES/MES) depends on the electron flux manifested by the bacteria. Thus it is very important to study the extra cellular electron transfer mechanisms so that losses can be minimized. Fundamental understanding of the specific electron transfer mechanism between biocatalyst and electrodes as well as among mixed microbial species is essential for improving the bioelectrogenic activity. The inherent low catalytic rate of microbes impounds limitation with respect to electron flux. To improve power density, electrogenic microbes with extracellular electron transfer capability need to be identified and enriched to limit some of the inherent issues with respect to anodic biocatalyst and electron transfer. Genetic engineering coupled with genome-scale metabolic modelling might also yield strains that can enhance bio-electrogenic activity. Further understanding of the microbe-electrode interactions is a pre-requisite to attempt the advancements in applications of MFC.

6.2. System

Still significant scientific inputs from multi-disciplinary areas are essentially required in the context of appropriate fuel cell materials viz., electrodes, membrane, etc. Electrodes govern both anodic as well as cathodic activity in fuel cell system. Characteristics, conductivity, surface properties, biocompatibility, surface modifications, etc. need to be comprehensively studied. Novel, simplified, inexpensive and durable electrode materials are essential. Research and development on low-cost separator materials with high proton transfer coefficient, low oxygen permeability and non-fouling nature will make system design economical. In the perspective of low fuel cell performance, improving terminal oxidation reaction i.e., cathode activity should be addressed. Majority of studies on MFC are still limited to bench scale. Focus on scaling up of technology is imperative in order to deliver it as a practical marketable technology. Stacking of fuel cells needs to be considered for higher power output through reduced mass transfer losses during up scaling. The MFCs should be configured and designed such that they can be easily integrated with current infrastructure.

6.3. Wastewater

Recent energy and environmental scenario is anticipating a paradigm shift from waste remediation towards energy generation. Reducing the treatment cost of waste and finding sustainable

ways to produce value-added products from waste treatment is current research interest in the context of biorefinery. Recently waste is being recognized as a potential feed-stock for valorization of various forms of bioenergy. Intrinsic advantage of using waste is its availability in huge quantities embedded with biodegradable organics that results in net positive energy gain in addition to remediation. Use of mixed microbial consortia as biocatalyst and wastewater as a substrate serves as a viable platform towards bioenergy generation with simultaneous treatment of waste.

6.4. Applications

In spite of the enduring challenges, if MFC keeps its pace in research and development, it is reasonable to believe that in the near future this platform technology will provide viable solutions to address many energy and environmental related issues. BES can be explored for bio-catalyzed electrochemical synthesis of various value added products in cathode chamber that would have commercial value by using waste as substrate, biological treated substrate or CO₂. BES can also be investigated for its application as biosensors. The inherent advantage existing with MFC to synchronize multiple bio-catalyzed electrochemical reactions in the advantage of waste treatment i.e. bioelectrochemical treatment is one potential application which needs considerable attention. However, MFC requires substantial research inputs in multi-disciplinary dimension to authenticate the fundamental mechanism towards multi-facet applications. Advances in materials and engineering are key driving factors which also play responsible role in this dimension.

Acknowledgments

The authors wish to thank Director, CSIR-IICT for support and encouragement in carrying out this work. This research was supported by Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India in the form of National Bioscience Award (BT/HRD/NBA/34/01/2012(vi)) and Council of Scientific and Industrial Research (CSIR), Government of India in the form of 12th Task Force Project (SETCA; CSC-063) GV duly acknowledge CSIR for providing RA fellowship.

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